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J. Nvota, A. Grom, A. Fáberová, D. Lamošová

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REACTION OF CHICKENS TO  
GRADUATED LENGTH OF EXPOSURE TO STRESS

J. Nvota, A. Grom, A. Fáberová, D. Lamošová

Institute of Physiology of Agricultural Animals, Slovak  
Academy of Sciences, Košice, Branch Ivanka pri Dunaji,  
and the Research Institute for Poultry Breeding, Ivanka

The problems of mutual relations between activity of individual components of the neuroendocrine system of animals in response to external stimuli assume, from the viewpoint of the adaptation process, an ever increasing importance. It has been documented that manipulation of one axis of the neuroendocrine system in animals can substantially affect the function of other axes. The study of interactions of neuroendocrine axes during stress or in new environmental situations is prospective in nature, as the obtained findings will serve to explain the adaptation mechanisms of animals in much closer detail. This applies primarily to young animals, in view of their impressibility in regards to new external stimuli. This study deals with the reaction of the axes hypophysis-adrenal gland and hypophysis-thyroid gland in young chickens exposed to graduated stress.

References list relatively many data regarding reactions of the axis hypophysis-adrenal gland in chickens to stress or to administration of ACTH, which, however, in the past often tended to be contradictory. [Jailer and Boas (1950), Howard and Constable (1958), Perek et al. (1960) did not detect in fowl any response to stress by depletion of adrenal ascorbic acid; Bates et al. (1940), Breitenbach (1962), and Siegel (1962) on the other hand established that fowl react to stress by depletion of adrenal ascorbic acid. Also Frankel

et al. (1967), Nvota et al. (1970), Bouille et al. (1969) detected an unambiguous response to stress by increasing the level of corticosterone in the plasma.]

We could find no data in references regarding the reaction of the axis hypophysis-thyroid gland in birds to mechanical stress noxae.

The mutual relation of activity in the axes hypophysis-adrenal gland and hypophysis-thyroid gland in adaptation reactions of animals to stress was pointed out in studies by Mikulaj et al. (1964), preceded by Hirvonen and Rasaenen (1954), and others. They determined that stress or administration of ACTH produces in rats increased activity in the axis hypophysis-adrenal gland while the activity of the axis hypophysis-thyroid gland decreases. Interactions between the activity of individual axes are probably formed already at the functional level of the hypophysis or the hypothalamus.

In our study we endeavored to establish basic data regarding the reaction of the axes hypophysis-adrenal gland and hypophysis-thyroid gland in young chickens to varying length of exposure to stress, as well as their interactions, so as to establish further findings regarding the progress of adaptation reactions in birds to new external stimuli.

#### Materiel and Methodology

In our experiments we used 30 chickens Arbor Acres 60 X Vantres, bred in the usual manner till age 60 days. Four days prior to the experiment we divided the chickens by random selection into 6 groups with 5 animals in each group, weighing and color-coding them in order to minimize disturbing them on the day of the experiment. The chickens in the 1st group served as controls and were decapitated first without any exposure. The remaining groups of chickens were next

exposed to stress by tying them to a base plate by textile bands, whereby the chickens of the 2nd group were decapitated and dissected after a half-hour immobilization, the 3rd group after 1 hour, the fourth group after 2 hours, and the 5th group after 4 hours. Each chicken in the 6th group was administered 5 mouse units of ACTH intraperitoneally and decapitated after 1 hour. Following decapitation, we determined for each chicken the weight of the adrenal gland, the level of corticosterone in adrenals and the blood plasma by fluoroscopy according to Van der Vies et al. (1960). We also determined the weight of the thyroid gland and its activity by histometry. The thyroid glands were then fixated in formalin and, after dehydration, embedded in paraffin. The 10 micron thick slices were stained by hematoxylin and koussin (Wolf, 1954). The relative contents of epithelium, colloid and stroma in the gland were determined by means of microscopic screen method according to Uotila and Kannas (1952), or Palkovits (1963) and Mitro et al. (1969), who dealt with and used this method in various modifications. Intensity of metabolism was determined by oxygen consumption in tissue cuts of the liver in vitro directly by Warburg's method according to Kleinzeller (1964). The results were statistically evaluated by the t-test according to Student. The curves in figures represent average mean values and average mean deviations arrived at in the experiment.

## Results

Relative Weight of Adrenal Glands. The chickens of the individual experimental groups showed in comparison with the control group no significant differences in the weight of the adrenal gland following exposure to stress (Fig. 1). We determined that the adrenal

gland did not react by changes in weight to the graduated length of exposure to stress.

The level of corticosterone in the adrenal glands of immobilized chickens increased upon exposure to stress, primarily in groups that were exposed to half-an-hour stress, but not significantly, and then gradually decreased in proportion to the length of exposure to stress (Fig. 2). In chickens that were exposed to 4 hours of stress, the level of corticosterone in the adrenal gland showed little difference from that of the control group. In the group that was administered ACTH, the corticosterone level in the adrenal gland increased the most and approached the level of statistical significance (P less than 0.10).

Fig. 1. Effects of graduated stress on changes in relative weights of adrenal glands in mg per 1,000 g of body weight.

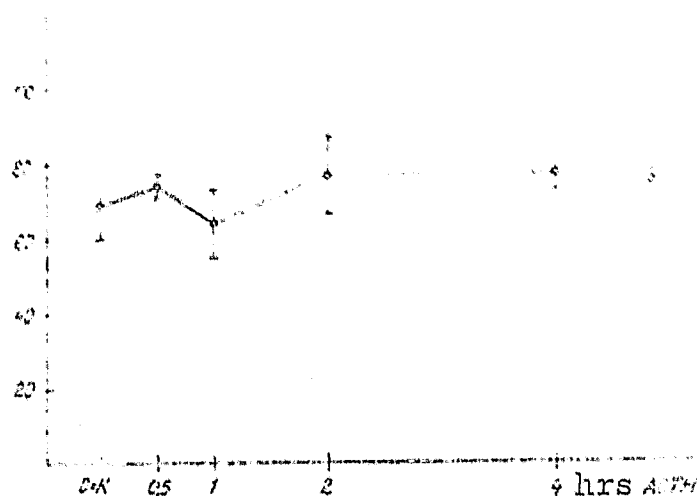


Fig. 1. Effects of graduated stress on changes in relative weights of adrenal glands in mg per 1,000 g of body weight.

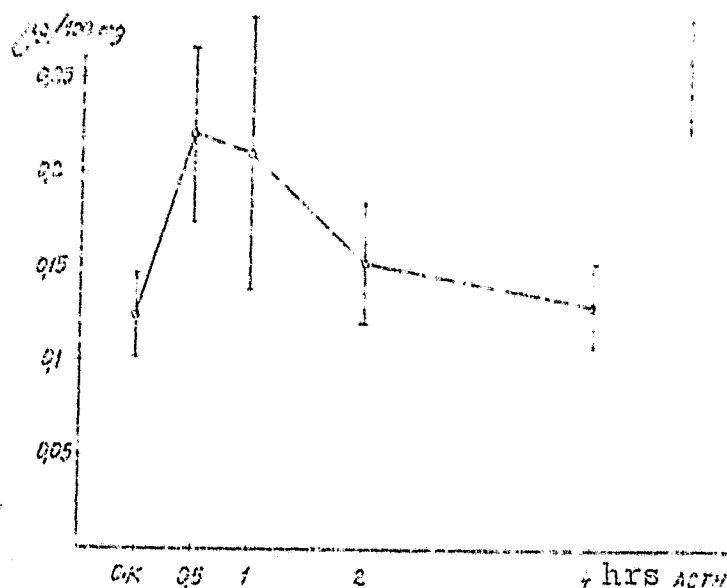


Fig. 2. Effects of graduated stress on corticosterone contents in the adrenal gland in micrograms per 100 milligrams of adrenal gland weight.

The level of corticosterone in plasma, similarly as the level of corticosterone in the adrenal gland, increased in the case of the immobilized groups (Fig. 3). The greatest, and statistically significant, increase occurred in the group exposed to 1/2 hour stress (P less than 0.01), then, as immobilization continued, the corticosterone level kept decreasing (after 1 hour P less than 0.05). Chickens exposed to 4 hours of stress showed the lowest level of corticosterone in their plasma, even though the difference in comparison with the control still was substantial, but not statistically significant. In chickens that were administered ACTH, the level of corticosterone in plasma increased very sharply, with a high degree of statistical significance (P less than 0.001).

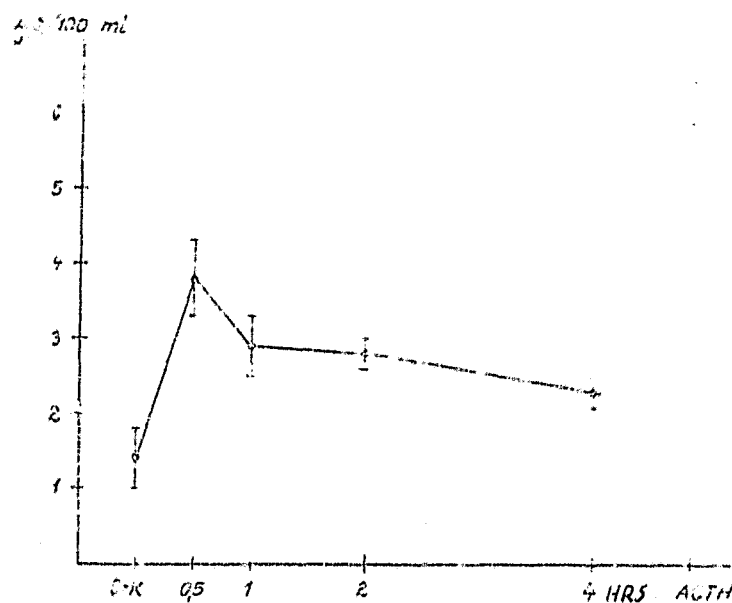


Fig. 3. Effects of Graduated Stress on the Level of Corticosterone in plasma, given in micrograms per 100 milliliters of plasma.

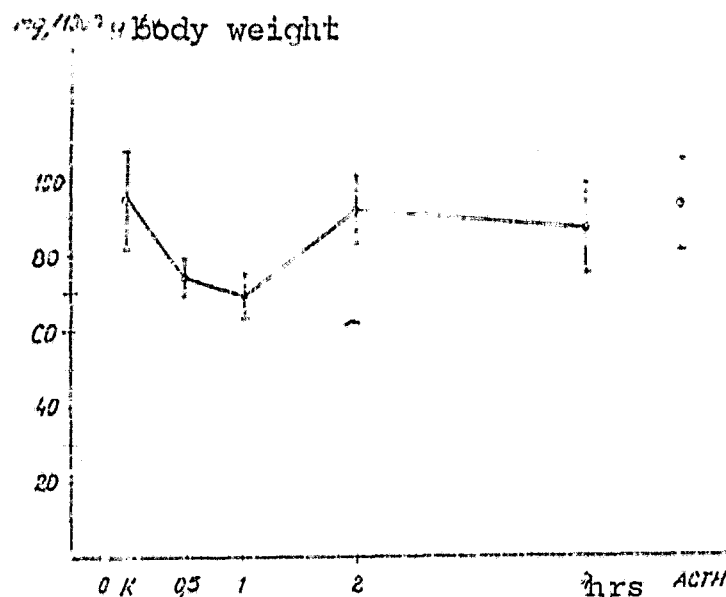


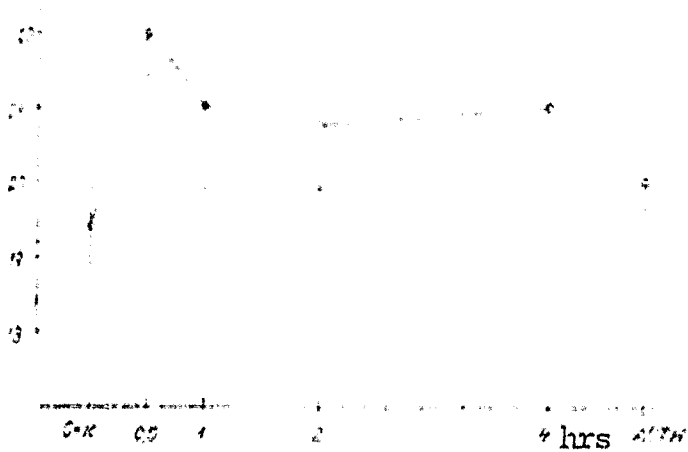
Fig. 4. Effects of Graduated Stress on Changes in the Relative Weight of Thyroid Glands, Given in mg per 1,000 g of body weight.

Relative Weight of Thyroid Glands. Thyroid glands of immobilized chickens reacted to stress by loss in weight. The reductions in relative weights of thyroid glands following exposure to stress were substantial (Fig. 4). The lowest and statistically significant ( $P$  less than 00.05) occurred in chickens exposed to stress for 1 hour. After that, the weight of the thyroid gland increased, but even after 4 hours it did not attain its initial level. In animals that were administered ACTH there was but a slight decrease in the weight of the thyroid gland.

The curve for the percentual contents of epithelium in the thyroid gland of chickens exposed to stress runs contrary to the curve for the relative weights of the thyroid glands (Fig. 5). The percentage of epithelium contents increased most in chickens exposed to 1/2 hour of stress. The difference was statistically significant



Fig. 5. Effects of Graduated Stress on Activity of Thyroid Glands, Determined Histometrically in Percent of Epithelium Area in Cross Sections of Thyroid Gland Follicles.



(P less than 0.02). In the course of subsequent immobilization the contents of epithelium decreased and ceased to change further. Following application of ACTH, there was but a slight and insignificant increase in the contents of epithelium in thyroid gland follicles as compared to the levels found in control animals.

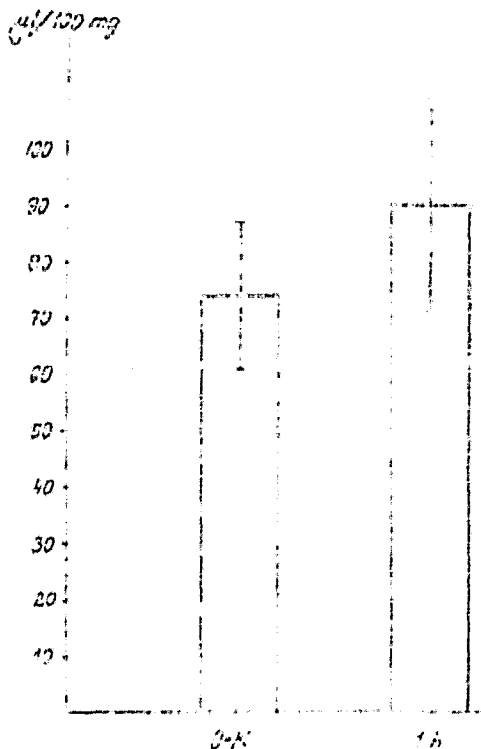


Fig. 6. Effects of Graduated Stress on Intensity of Metabolism, Expressed by oxygen consumption in microliters per 100 milligrams of Liver Tissue.

Oxygen consumption, determined in tissue cuts of liver in vitro for chickens exposed to 1 hour of stress did detectably increase in comparison with the oxygen consumption of the control group, but not enough to be of statistical significance.

### Discussion

Our findings regarding the reactions of chickens to stress which became manifest in increased activity of the axis hypophysis-adrenal gland, as well as the axis hypophysis-thyroid gland, differ from the results arrived at by Mikulaj et al. (1964), Hirvonen and Raesaenen (1964), Epstein (1953) and others using rats in their experiments. The latter do respond to acute stress by increased activity of the axis hypophysis-adrenal gland, but, on the other hand, by a decrease in the activity of the axis hypophysis-thyroid gland. As a rule, the secretion of TSH in rats decreases whenever the level of ACTH is increasing, probably due to interaction in neurosecretion of hypothalamic effects of CRF and TRF. According to our findings in chickens, the axis hypophysis-thyroid gland also plays an active role in stress reactions to external stimuli. The findings simultaneously point out the difference in reaction of phylogenetically remote groups.

Changes in activity of the axis hypophysis-adrenal gland and hypophysis-thyroid gland progressed simultaneously during immobilization. Highest increases in activity were detected after half-hour exposures to stress. In the course of prolonged immobilization, the studied parameters reversed to their initial values.

The lack of uniform data in references regarding the reaction of the axis hypophysis-adrenal gland to administration of ACTH, as well stress in fowl, was due, primarily in the past, to different

methodological approaches. Jailer and Boas (1950), Howard and Constable (1958), Perek et al. (1960) did not find the answer to stress by increased depletion of adrenal ascorbic acid. Bates et al. (1940), Breitenbach (1962), Siegel (1962) found response in adrenal glands of chickens to administration of ACTH by increased depletion of ascorbic acid. Newer studies that dealt with corticosterone in plasma (Frankel et al, 1967; Nvota et al., 1970; Bouille et al., 1969) showed that stress in birds unambiguously produces an increase in the level of corticosterone in blood plasma. Histometric determination of the amount of epithelium of the colloid and stoma undertaken in this experiment is, according to studies by Palkovits (1963) and Mitro et al. (1969) is a good indicator of activity. We found no data in any references dealing with the relation between the activity of the studied neuroendocrine axes in stress situations in birds to enable us to make any comparisons.

### Summary

The study sought to establish how chickens Arbor Acres 60x Vantres at age 60 days react to 1/2, 1, 2, 4 hour immobilization and to administration of ACTH. The reaction was monitored by determining the activity of the axis hypophysis-adrenal gland on the basis of changes in the relative weight of the adrenal gland, the level of corticosterone in adrenals and in plasma, as well as by determining the activity of the axis hypophysis-thyroid gland on the basis of changes in the relative weight of the thyroid gland, its percentage of epithelium and oxygen consumption in liver tissue. Young chicks showed highest response after a half-an-hour exposure to stress along both the studied neuroendocrine axes. During prolonged stress

the response weakened and after 4 hours most measured values gradually approximated their initial levels. The responses of both the axes were synchronized. Response of both studied endocrine axes in birds progressed in the same direction, i.e., increased activity of both the adrenal and thyroid glands. This response differs from that determined on laboratory rats, in which an increased activity of the axis hypophysis-adrenal gland is accompanied by a decrease in the activity of the axis hypophysis-thyroid gland.

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Authors' Address:

Engr Ján Nvota, Cand Sci.; Dr Anna Fáberová, Cand Sci; Engr dalma Lamošová, Institute of Physiology of Agricultural Animals of the Slovak Academy of Sciences, Field Station in Ivanka near Danube; Prof. Anton Grom, Cand Sci, Research Institute for Poultry Breeding, Ivanka near the Danube River.